Results:

Due to the differences in seeding methods and results at each site, the results are presented by individual site with a summary analysis at the end. The methodology for seed dispersal, seed bags deployment, and monitoring are the same for each site. However, due to differences in bathymetry, currents, and obstructions, the size of each area will not be uniform.

Seed collection

2003: Reproductive shoots from healthy eelgrass beds containing mature seeds were collected manually in Tangier Sound. Harvesting took place on May 20, 23, and 27-30 and yielded 2.3 million seeds, 250,000 of which were viable for broadcast.

2004: A mechanical harvest boat was utilized to increase the efficiency and amount of reproductive material collected. From May 24 to June 4, 2004, seeds were collected from donor beds in the Little Annemessex River. In nine cutting days the mechanical harvester collected approximately 71.92 L of eelgrass reproductive material. In 2004, the portion of reproductive material transported to Piney Point for seed extraction yielded 15.12 million seeds. After the seed processing and storage process was complete, 7% of the collected seeds (or 1,058,400 seeds) were viable for broadcast.

2005: Reproductive material was harvested from the Little Annemessex River and the mouth of Acre Creek (Big Annemessex River) from May 23 to June 8, 2005 (Fig. 5). The harvest machine collected approximately 109.5 L of eelgrass seeds from 21.6 acres of eelgrass beds. Seed count estimates were made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material. Replicate 2 ml samples of seed material were analyzed for the number of viable seeds. The total number of seeds harvested was calculated as the sum of the number of seeds per ml (113/ml) and the total volume of seeds collected (109.5 L). Based on this calculation, the portion of reproductive material transported to Piney Point for seed extraction yielded 12,373,500 seeds. An estimate of the number of viable seeds was also determined as the sum of the number of viable seeds (68 viable seeds/ml) and the total volume. Using this calculation, there were an estimated 7,446,000 viable seeds, 60 percent of the total number of seeds collected, after processing was through. After storage of the seeds throughout the summer, there were a total of 2,527,000 viable seeds.

In spring 2003, 2.3 million seeds were gathered by hand using snorkeling and SCUBA equipment with a majority of the seeds coming from Sinepuxent Bay. From that harvest, 250, 000 seeds were available for harvest, giving a yield of

11%. The seeds were taken to Piney Point, where they were separated and maintained in storage containers for the fall 2003 hand dispersal. The seed material harvested in this fashion contained more seeds per bag because the divers/snorkelers could differentiate the reproductive shoots. While the seeds counts per bag were considerably higher than later years, this method required more than 500 man hours and was extremely time consuming, so improvements were made in subsequent harvests.
Whether for use in fall seed broadcasts or spring seed bags, it is necessary to know the number of viable seeds in order to achieve predetermined seeding densities and to determine the subsequent recruitment rate (number of seedlings/number of viable seeds distributed). For the spring seed bag method, the number of seeds placed into seed bags was estimated by counting seeds in four 1L subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material. This gives us an estimate of the total number of seeds dispersed using the seed bag method. However, because we never extract the seeds from the spathes to analyze each of them individually there is no direct measure of the number of viable seeds vs. dead or non-viable seeds. Therefore, recruitment is the number of seedlings recruited/the total number of seeds distributed.

Seeds to be used for fall seed broadcast are separated from reproductive material at the Piney Point facility. Two methods were used to count seeds to be used for fall seed broadcasts, one before the seeds separated from reproductive material, and one after seeds had been processed. In 2005, the portion of reproductive material transported to Piney Point for seed extraction yielded between 12 and 32 million seeds. The 32,806,200 seed estimate was determined shortly after collection by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L (210 seeds/L) by the total volume of harvested material (149,800 L). This gives us an estimate of the number of seeds dispersed using the seed bag method because we never extract the seeds from the spathes to count them directly. The 12,373,500 seed estimate was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material.

The total number of seeds at Piney Point in 2005 was calculated using the number of seeds per ml (113/ml) compared to the total volume of seeds collected (109.5 L). An estimate of the number of viable seeds was also determined using replicate 2 ml samples of seed material. The number of viable seeds (determined using the squeeze test) per mL was compared to the total seed volume. Using this calculation, the 68 viable seeds/ml can be extrapolated to predict 7,446,000 million total viable seeds.

The first seed count method estimates the totally number of seeds collected. However, because this method does not account for seed losses through any number of processes it may not accurately reflect the true number of seeds available for broadcasting. While the other method estimates number of seeds and determines the viability of seeds, it too has some sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability in total seed number between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates and thus can't determine the best estimate.

Seed Dispersal and Test Plantings

Seeds were dispersed by hand in 2003. Seven rings of 5m each were seeded with 50,000 seeds per ring (Fig. 3). This site was adjacent to the Woodrow Wilson Bridge Mitigation project, allowing for a side by side comparison of the effectiveness of planting seeds vs. adult shoots.

In 2004 and 2005, seeds were dispersed in the spring and fall. In 2004, 2,400,000 seeds were dispersed in the spring and 262,500 in the fall. In 2005, 4,510,000 seeds were dispersed in the spring and 400,000 seeds were dispersed in the fall. Listed below is a summary of the seeds dispersed, the method of dispersal, along with field observations made during the monitoring for each site

Piney Point

DNR biologists used the ring method developed by Orth (Personal Communication) to disperse the 250,000 viable seeds available at the time of dispersal in 2003. Adjacent to the 2003 hand broadcast areas, 150,000 seeds were broadcast in a 0.5 acre plot in fall 2004 (Fig. 3).

Table 1. Piney Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Piney Point	Seed Broadcast	05/12/2005	0.5	150,000	0	0
		8/1/2005			0	0

		11/3/2005			0	0

St. George Island

In spring 2004, seed bags containing 605,000 seeds were dispersed in a 5 acre plot (Fig. 3). The site was monitored for the first time on May 12, 2005 and there were 567 eelgrass plants observed per acre, with an estimated 2,835 eelgrass plants in the entire plot.

In the fall of 2004, 75,000 seeds were dispersed by machine broadcast in a 0.3 acre plot. The fall seeding area had 586 eelgrass plants per acre on May 12, 2005 for an estimated 147 plants in the plot.

Test plantings placed at each site in November 2004 were monitored on the same dates. In May 2005, an average of 55 plants were observed among the three test plots at St. George Island, yielding an 86% initial planting success rate. In August, 6% of the plants remained, half of which survived through November 2005.

Table 2. St. George Island Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
St. George Island	Seed bags	05/12/2005	5	605,000	567	2835
		08/1/2005			369	1985
		11/3/2005			45	213
	Machine Broadcast	05/12/2005	0.25	75,000	586	147
		08/1/2005			1246	312
		11/3/2005			37	9

Sage Point

In 2004, there was only spring seed bag dispersal at this site. There were two sites, each with 605,000 seeds spread over 5 acre plots (Fig. 3). Field observations made by biologists identified large amounts of widgeon grass, snails, and live oysters on the bottom.

Table 3. Sage Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Sage Point	Seed bags	05/12/2005	5	605,000	509	2545
		08/1/2005			0	0
		11/3/2005			0	0
	Seed bags	05/12/2005	5	605,000	128	641
		08/1/2005			0	0
		11/3/2005			0	0

Test plantings placed at this site in November 2004 were monitored on the same dates. In May 2005, an average of 52 plants was observed among the three test plots at Sage Point, yielding an 81% initial planting success rate. In August and November of 2005, no plants were observed.

<u>Cherryfield Point</u>

In 2004, there was a spring seed bag and fall seed broadcast at this site. In the spring, two adjacent 2.5 acre plots were seeded with seed bags with 275,000 seeds dispersed in each plot (550,000 total, Fig. 3).

Table 4. Cherryfield Point Seeding Results

Site	Seeding Method	Sampling Date (2005)	Plot Size (Acres)	Number of seeds	Plants per acre	Estimated plants in plot
Cherryfield Point	Seed bags	05/12/2005	2.5	275,000	437	1092
		08/1/2005			16	39
		11/3/2005			0	0
	Seed bags	05/12/2005	2.5	275,000	32	50
		08/1/2005			0	0
		11/3/2005			0	0
	Machine Broadcast	05/12/2005	0.25	37,500	0	0
		08/1/2005			0	0
		11/3/2005			0	0

Test plantings placed at this site in November 2004 were monitored on the same dates. In May 2005, an average of 11 plants was observed among the three test plots at Cherryfield Point, yielding a 17% initial planting success rate. In August and November, no plants were observed.

Water quality

The SAV strategy calls not only for large scale SAV restoration projects, but also for assessment of the associated habitat conditions in order to evaluate reason for success or failure and to improve the likelihood of success for future projects. In keeping with the requirement of this strategy, long term, fixed and continuous water quality monitoring was conducted for 2004 and 2005. Data from the continuous monitoring stations and the water quality mapping cruises were analyzed to explain the seed germination and plant survival results. In addition, the 2004 data will be compared to the nearby Potomac River Mainstem cruise 20 year data record to assess if conditions during this project period were anomalous.

Light availability and temperature are the two most critical water quality parameters for Z. marina (Stankelis, 2003). In the Chesapeake Bay, there is a well-documented bimodal eelgrass growth pattern with primary growing season beginning when temperatures rise above 10oC with a peak in biomass occurring in late May to early June (Orth, review). A second, less dramatic growing season occurs in mid-September and continues until water temperatures drop below 10oC sometime in November. Increasing light attenuation and water temperature (above 25oC) later in June cause decreased growth and leaf defoliation (Moore et al. 1996; 1997).

The continuous monitoring data provide an in depth record for some of the parameters (turbidity, temperature) that affect SAV during the summer season. Four graphs that summarize data collected for 2004 and 2005 at the Piney Point and Sage Point water quality monitoring stations (Fig. 15 & 16).

Water quality mapping and Potomac River Mainstem cruises are marked on the graphs (Fig. 17-19). The red line indicates a Nephelometric Turbidity Unit (NTU) of 5.38, the turbidity compensation depth, (the water depth above which plants at 1M deep will not receive the light necessary to carry out basic metabolic functions). On the 2005 graphs, dates when DNR monitored the plants at each site were also marked. The Potomac River experienced turbidity values above 5.38 for most of the summer. The actual values on a particular day are not as important as the number of consecutive days these values were above the turbidity compensation depth.

<u>Piney Point</u>

Data was recorded at the Piney Point Water Quality monitor from March through the end of October 2004. In 2004, the turbidity values exceeded the 5.38 NTU maximum for 18% of the year and exceeded the maximum for 18% of the SAV growing season. In 2004, the records indicate that temperature did not exceed 30^{0}C for the entire data record. In 2005, the values exceeded the 5.38 maximum for 7% of the year and also for 7% of the SAV growing season. In 2005, the records for temperature data show that temperature exceeded this 30^{0}C for 2.6% of the year.

<u>Sage Point</u>

In 2004, the Sage Point continuous monitor showed that the turbidity exceeded the 5.38 maximum 27% of the year, and exceeded that value for 26% of the SAV growing season. In 2004, the records indicate that temperature did not exceed 30^{0}C for the entire data record. Turbidity was lower in 2005, and only exceeds the 5.38 NTU limit for 17% of the year. Turbidity during the SAV growing season also exceeded the limit 17% of the time. In 2005, the records for temperature data show that temperature exceeded this 30^{0}C for 2.6% of the year at Sage Point.

Fluorescence was another parameter monitored by the continuous monitor stations. Correlation values were determined for turbidity from the 2004 and 2005 data sets. At the Piney Point Site, the Pearson Correlation Coefficient was 0.08175 (P Value= .0001, N = 17707) in 2004 and 2005 yielded a 0.07070 Pearson Correlation Coefficient (P Value= .0001, N = 18441). At Sage Point in 2004, the data yielded a -0.01153 Pearson Correlation Coefficient (P Value = 0.1072, N = 19524). The data for 2005 yielded a 0.14798 Pearson Correlation Coefficient (P Value =.0001, N = 16674).

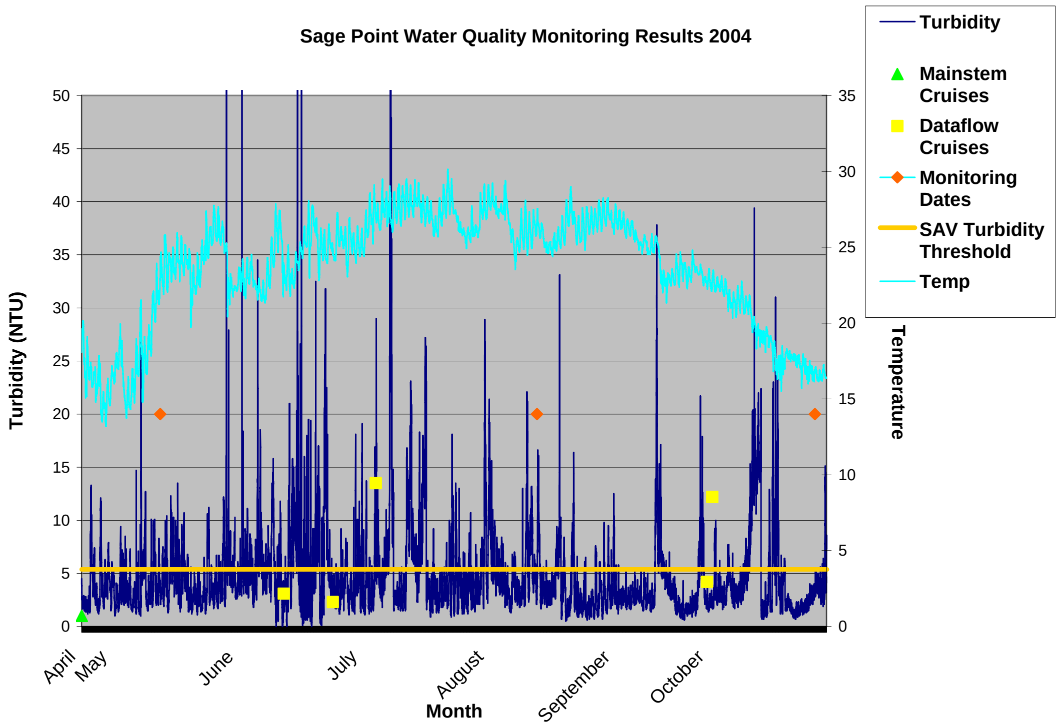
Water Quality Mapping

Water quality mapping data are also represented as a map due to the large number of data points. Cruise pattern data are interpolated to provide graphs indicating parameters levels. Each graph shows a picture of the turbidity conditions present in the Potomac for each month. The data show a picture of the water quality for the whole river at a fixed point in time, and can be used in conjunction with continuous monitoring data to identify small scale differences.

Water quality mapping was conducted monthly throughout the eelgrass growing season (March-November) throughout the lower portion of the river. Turbidity data were compiled for 2004 and 2005. Water quality mapping cruises were conducted and turbidity data were analyzed from April to October 2004 and April to October 2005, with 2 cruises in each month in 2005. In 2004, turbidity peaked in June, with values between 5-7.5 NTU's. In August and September 2004, there was a spike in turbidity upriver from the St. George Island site and there were patches of high turbidity around the restoration site. In 2005, there was a similar spike in turbidity in June, August and September at St. George Island. The rest of the sites remained unaffected, with values lower than 2.5 for most of the year.

Finally, data from the Mainstem bay monitoring cruises collected Secchi depth from 2003-2005. Due to the large amount of data points, these data are represented graphically in relation to the 20 year average. The graphs compare the Secchi depth readings collected for each year, compare it to the mean for the prior years, and show the range of data over the 20 year period.

Figure 15. Continuous monitoring data for Sage Point, 2004 and 2005.



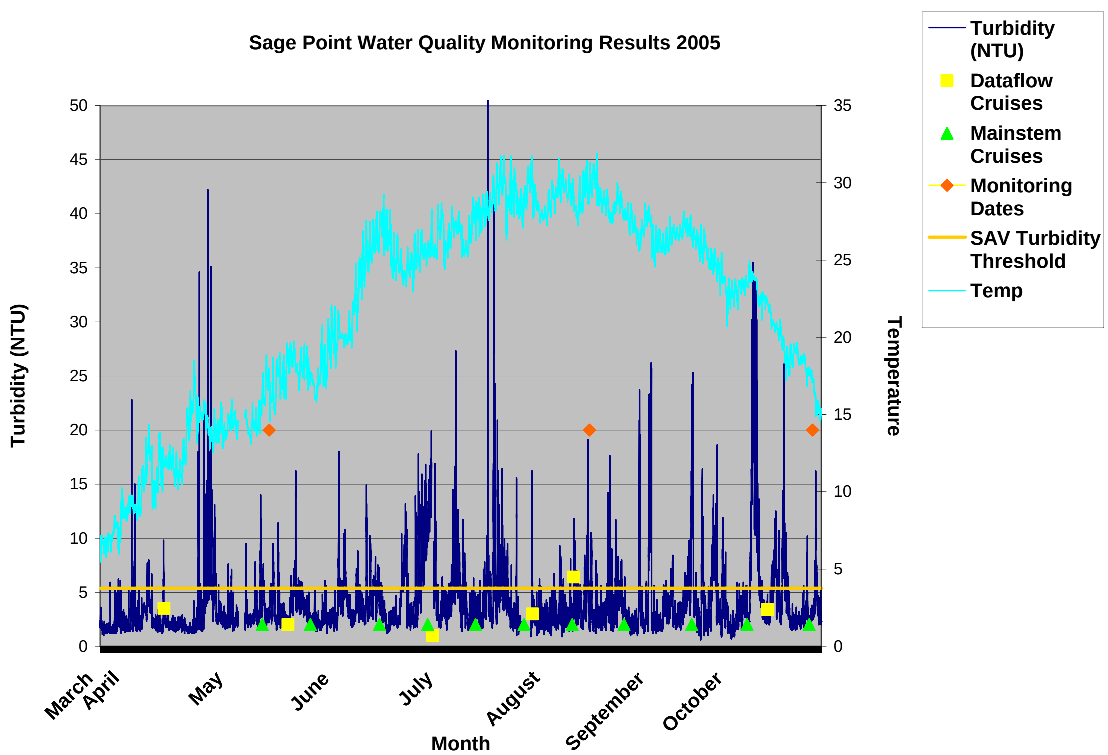
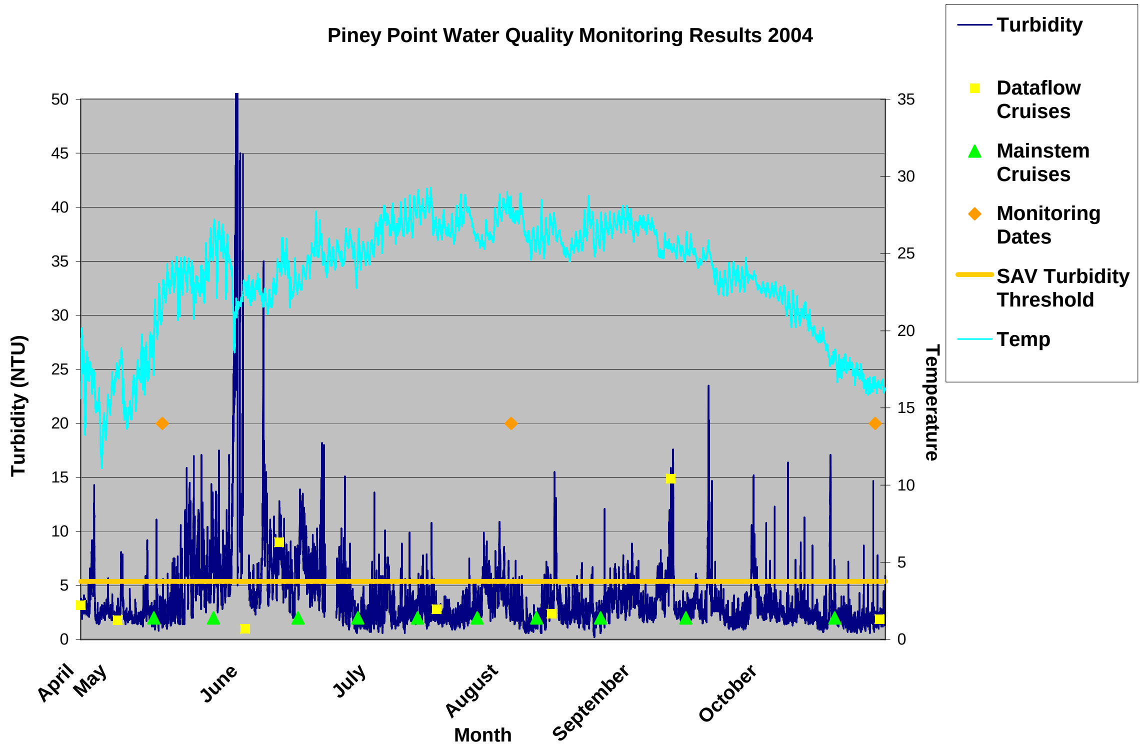


Figure 16. Continuous monitoring data for Piney Point, 2004 and 2005.



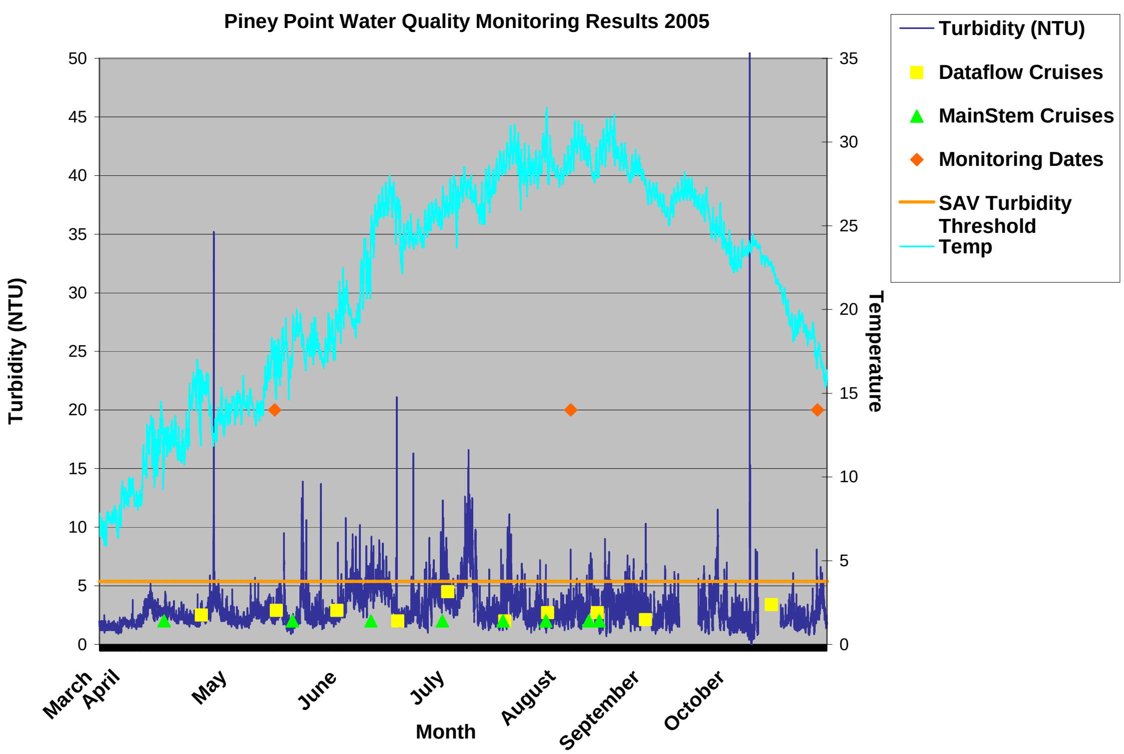


Figure 17. Turbidity data from 2004 Water Quality Monitoring Cruises.

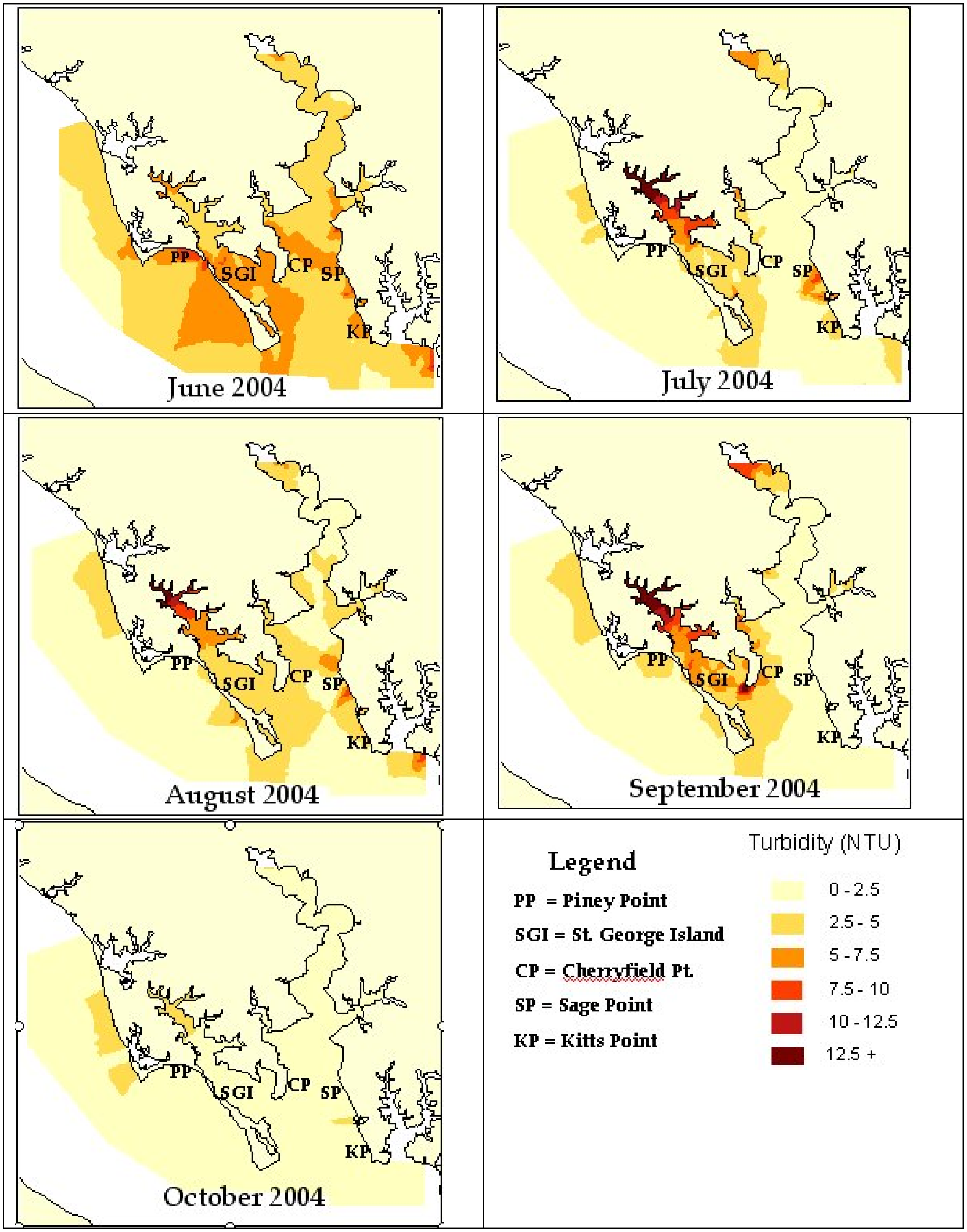


Figure 18. Turbidity data from water quality mapping cruises, 2005.

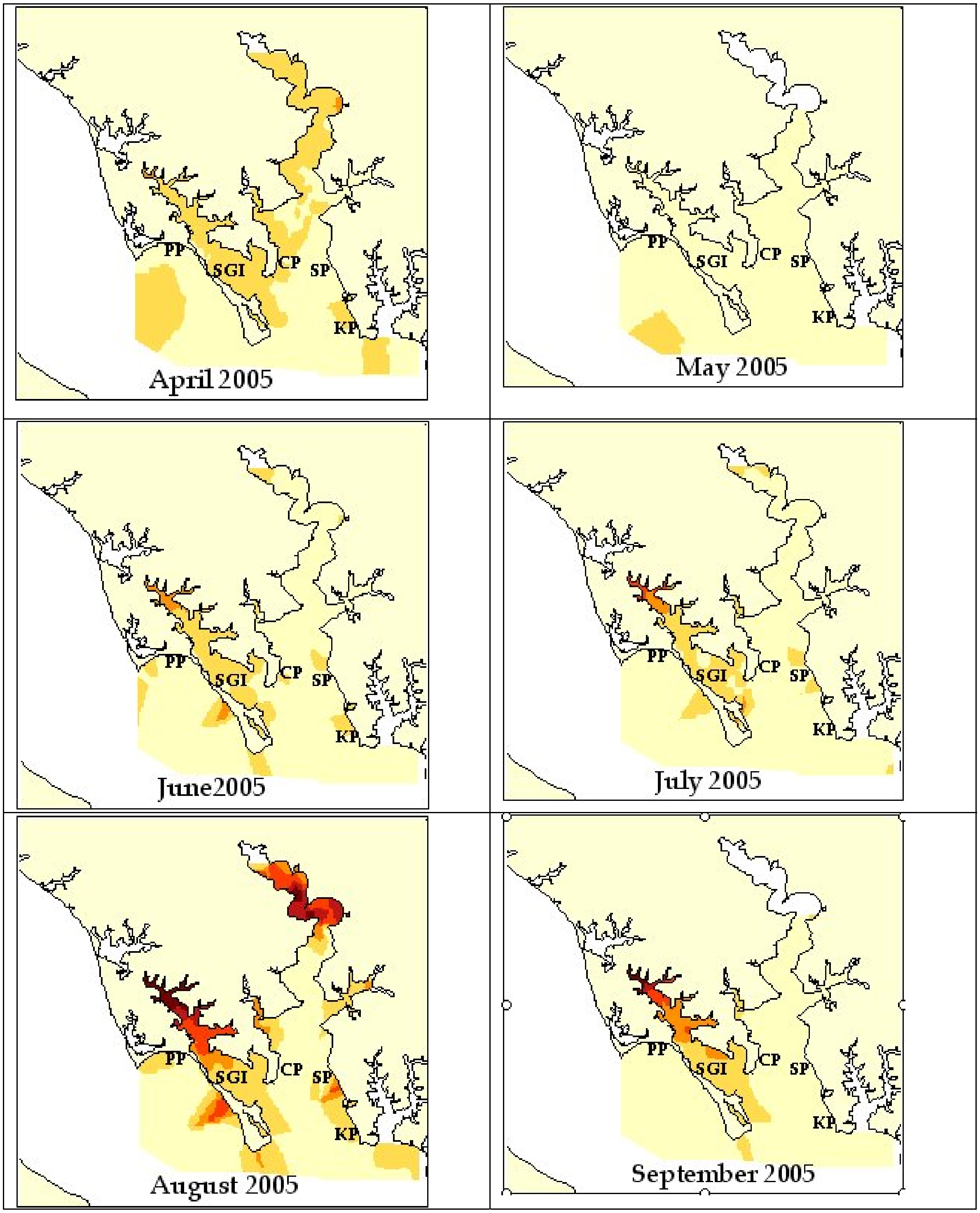


Figure 18 (cont'd). Turbidity data from 2005 water quality mapping cruises

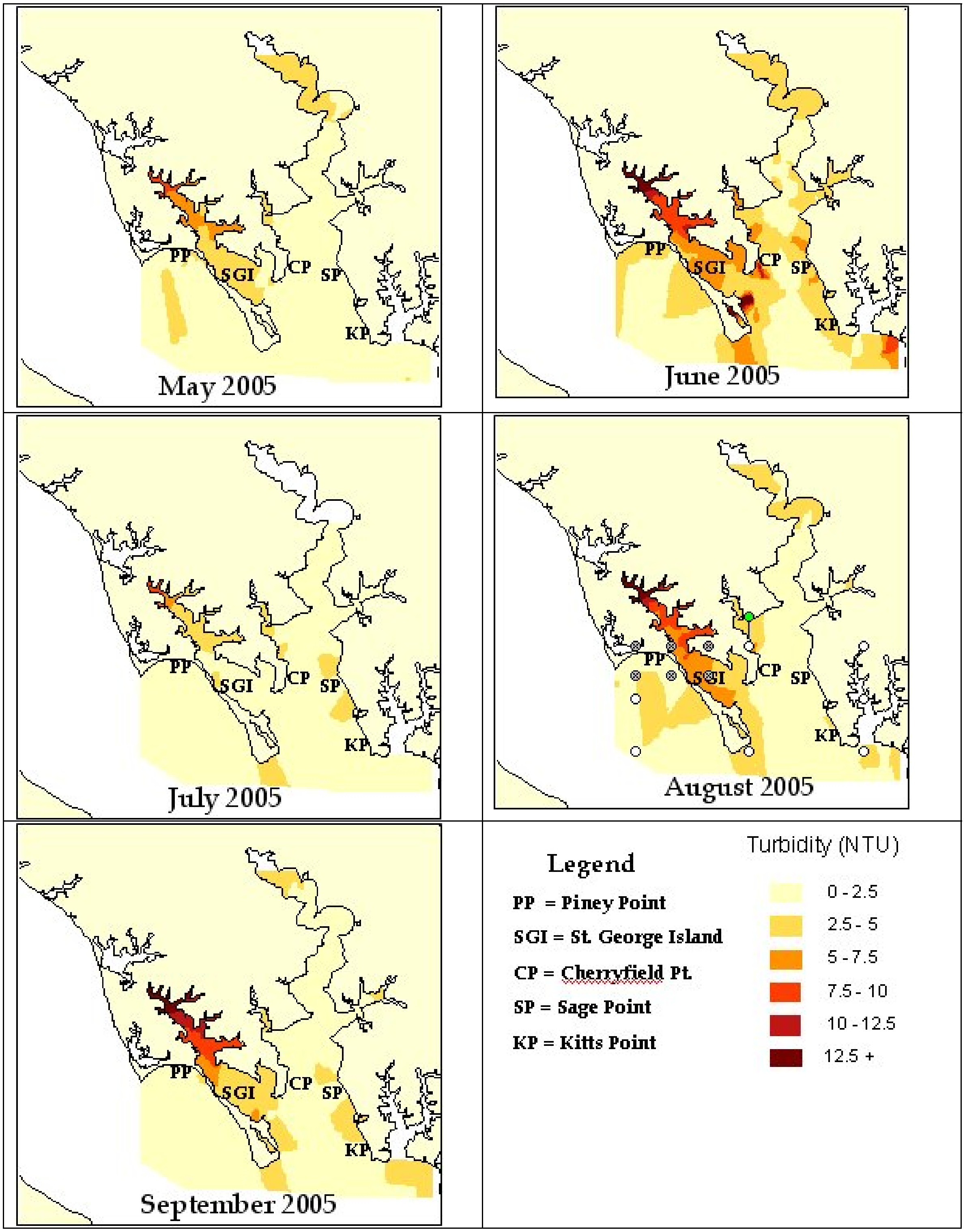


Figure 19. 2003-2005 Secchi Depth at Point Lookout Monitoring Station

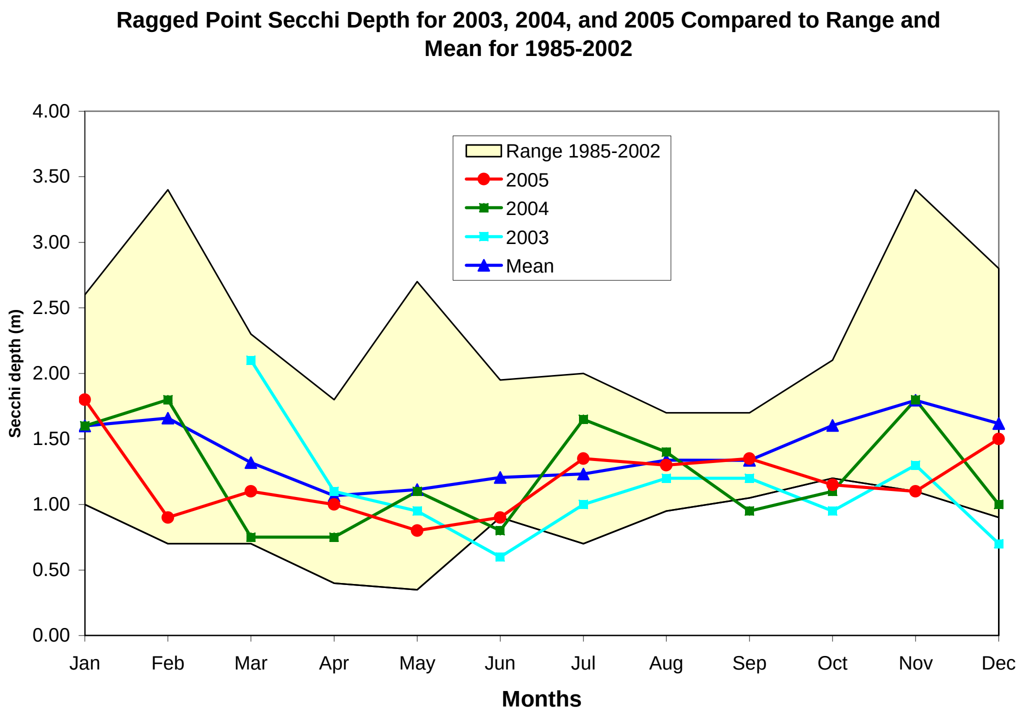


Table 5. Water quality mapping data for 2004, Sage Point and Piney Point

Site	water quality mapping CRUISE	Turbidity Value(NTU's)		Site	water quality mapping CRUISE	Turbidity Value(NTU's)
Piney Pt.	May	2.5-5		**Sage Pt.**	May	2.5-5
	June	7.5-10			June	5-7.5
	July	0-2.5			July	5-7.5
	August	0-2.5			August	2.5-5
	September	2.5-5			September	0-2.5

Table 6. water quality mapping Cruise Data for 2005, Sage Point and Piney Point

Site	water quality mapping CRUISE	Turbidity Value(NTU's)		Site	water quality mapping CRUISE	Turbidity Value(NTU's)
Piney Pt.	May	0-2.5		**Sage Pt.**	May	0-2.5
	June	2.5-5			June	2.5-5
	July	0-2.5			July	0-2.5
	August	2.5-5			August	0-2.5
	September	0-2.5			September	0-2.5

Mitigation Plantings

Survival was monitored at one month, six months and twelve months, and was estimated as a percentage of the original planting that survived. Of the 2003 plantings, at one month the sago pondweed had a 10.4% survival rate, widgeon grass had a 6.9% survival rate, and eelgrass had a 33.7% survival rate. At 6 months, eelgrass had a 26.7% survival rate, but the other two species did not survive. After 12 months, 9% of the eelgrass survived.

For 2004, at one month 3% of the sago pondweed, 9.1% of the widgeon grass, and 50.2% of the eelgrass survived. At 6 months, 3% of the sago pondweed, 9% of the widgeon grass, and 37.9% of the eelgrass survived. After 12 months none of the plants remained.

At the Sage Point site in 2004, at one month sago pondweed had a 10.1% survival rate and widgeon grass had a 0.1% survival rate. After 6 months no plants survived.

Seeding method cost comparison

In the spring of 2004, 20 acres were covered with seed bags, with approximately 2.4 million seeds distributed. In the fall, 1 acre was seeded by machine broadcast, distributing 262,000 seeds. The estimated number of plants for the spring seeding was 7,193, and the estimate for the fall was 147.

The total cost for seeding one acre was calculated by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. The total cost for each method was divided by the total number of successfully recruited seedlings to determine a ratio of cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods.

The cost per seed put out in Maryland for 2004 was $0.02 for the spring seed bag method and $0.34 for the fall seed broadcast. The total cost for seeding one acre was determined by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The cost for restoring one acre was determined to be $4,473 for the spring seed bag method and $67,085 for the fall seed broadcast method.

The spring seed bag method yielded 7,193 seedlings across all spring seed bag sites locations out of 2.4 million seeds broadcast, a recruitment success rate of 0.3%. The fall seed broadcast method yielded 147 seedlings across all fall seed broadcasts locations out of 262,000 seeds were dispersed, a recruitment success rate of 0.06%.

Each seedling (7,193) successfully recruited using the spring seed bag method cost $1.70. Each seedling (147) successfully recruited using the fall seed broadcast method is $363.89. For the purpose of cost comparison between methods, site selection and monitoring costs were not included. At the Piney Point site, RK&K engineers planted 15,000 eelgrass PUs, 1,600 widgeon grass PUs and 946 PUs of sago pondweed plants. Their cost per plant was $4.70.